JOURNAL OF TROPICAL BIOLOGY AND CONSERVATION 8 : 83 – 88, 2011

Short Communication

New distribution record of the Ashy Roundleaf Bat *Hipposideros cineraceus* Blyth 1853 in Sarawak, Malaysian Borneo: Conservation implications

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ABSTRACT. Here we report a new distribution record of the Ashy Roundleaf bat (*Hipposideros cineraceus*) from Mount Murud, Sarawak. These specimens were initially assigned to *H. ater*, in the field using external attributes and measurements. As these specimens' external measurements overlaps other morphologically similar *bicolor* species group (e.g., *H. bicolor*, *H. cineraceus* and *H. dyacorum*), the recognition of these specimens remains uncertain. We employed morphometric hierarchical cluster analysis and molecular DNA sequencing techniques to provide species level discrimination between other similar *bicolor* species group individuals. Results from the analyses suggest that specimens assigned as *H. ater* were misidentified, and comparisons with other related species description provide evidence for the recognition of *H. cineraceus*. Subsequently, this documents the first record of *H. cineraceus* distribution in Mount Murud, Sarawak. Specimens studied here diverged genetically (5 %) in cytochrome *b* gene from those in Peninsular Malaysia, suggesting that *H. cineraceus* individuals in Borneo or at least in Sarawak may represent a different evolutionary lineage. We propose the revision of the *H. cineraceus* conservation status, especially for those in Borneo to be changed to endangered species status, given the scarcity of their distribution and genetic divergence. Further studies incorporating specimens from other populations from the Asian mainland and Borneo may provide insights in reevaluating the taxonomic status and their specific conservation status in this *H. cineraceus* complex.

Keywords: Sarawak, Cluster analysis, Cytochrome-*b*, Conservation status

Between 29 May and 7 June 2005, a field survey was conducted at Mount Murud, Sarawak (Figure 1). Three adult male specimens assigned as *Hipposideros ater* were collected using four-bank harp traps by Anwarali *et al.* (2007a) at Raven's Court, Mount Murud (04°09'22.7N; 115°46'58.2E, 1335 m above sea level). These specimens were identified in the field using external characters: forearm length, tibia length, tail length, noseleaf structure and pelage coloration following Payne *et al.* (1985). The first record for *H. ater* in Sarawak was recorded by Abdullah *et al.* (2003) at Jambusan Cave, Bau followed by Anwarali *et al.* (2007b) in Bako National Park. Both of these reports have only used external measurements to establish species identification. As external

Received 6 December 2010. Revision accepted 9 February 2011

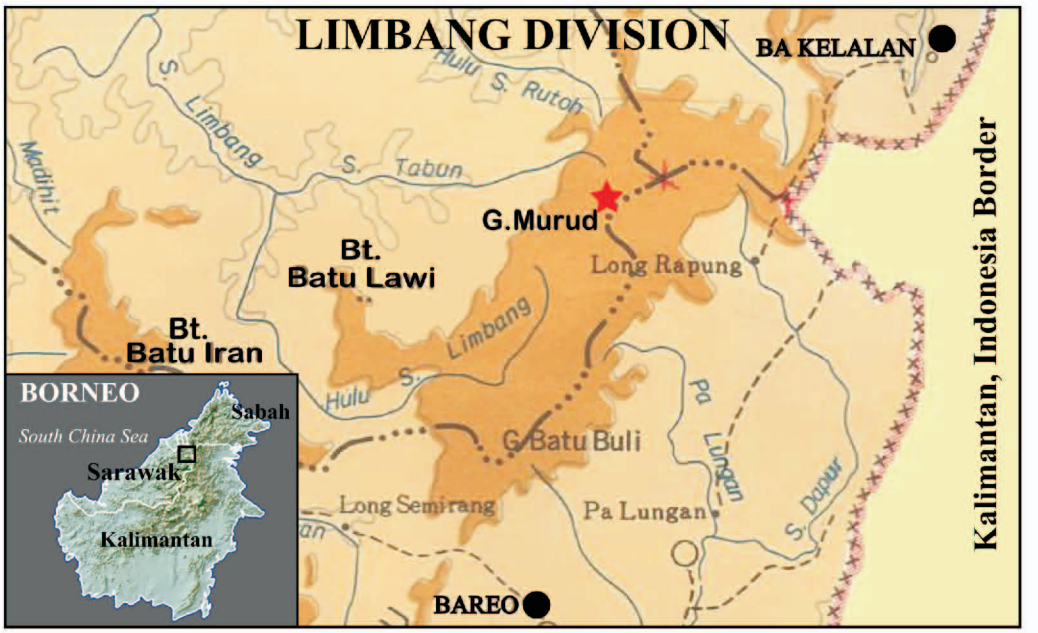

Figure 1. Map of Mount Murud, Sarawak (adapted and modified from Anwarali *et al.,* 2007a).

measurements of *H. ater* overlaps with other *bicolor* species group (Tate, 1941), identification of previous *H. ater* as well as those from Mount Murud remained uncertain.

Therefore, these specimens were morphologically analysed using 27 morphometric characters and compared with other morphologically similar *bicolor* species group individuals: *H. ater* (n=5), *H. bicolor* (n=6), *H. cineraceus* (n=3) and *H. dyacorum* (n=5). Both cranial and external measurements were taken from specimens deposited at the Universiti Malaysia Sarawak (UNIMAS) Zoological Museum and these were compared with three other male specimens from Peninsular Malaysia (Table 1). We extend our comparison with other published records in Borneo, including Hill (1963), Philips (1967), Payne *et al.* (1985), Koopman (1994), Kingston *et al.* (2006), and Suyanto & Struebig (2007). Partial mitochondrial DNA (413 bp) of cytochrome *b* (cyt *b*) gene (GludG-L: 5'-TGACT TGAAR AACCA YCGTT G-3'; CB2-H: 5'-CCCTC AGAAT GATAT TTGTC CTCA-3' (Palumbi *et al.*, 1991)) were produced and genetically analysed (refer Sazali *et al.*, 2011). Genetic divergences were calculated using the Kimura 2-parameter model (Kimura, 1980) and compared within Mount Murud specimens and other *bicolor* species individuals from Borneo to establish interspecific discriminations within the geographic area.

Results from both analyses were congruent and revealed that the three male specimens from Mount Murud were misidentified, suggesting the recognition of *H. cineraceus* Blyth, 1853 following cranial description by Hill (1963). *Hipposideros cineraceus* can be distinguished from *H. ater* by its slender and slightly smaller skull, delicate zygomata which lacks a jugal projection and a less reduced, unextruded anterior upper premolar (*sensu* Hill, 1963). Both morphometric hierarchical cluster (Figure 2) and molecular genetic analyses (Sazali *et al.*, 2011) suggest the groupings of these specimens with other individuals

Table 1. Comparison of external body and skull measurements of three male *H. cineraceus* from Raven's Court, Mount Murud, Sarawak with *H. ater*, *H. bicolor* and *H. dyacorum* analysed from UNIMAS Zoological Musuem* as well as with other authors' measurements compiled by Sazali (2009).

Species / Character	*H. cineraceus*	*H. ater*	*H. bicolor*	*H. dyacorum*
FA	35.04-36.99*, 36.5-38.5 (1), 34.0 (3), 36.0-40.5 (a), 32.0-36.5 (b), 32.0-37.0 (c), 35.0-38.0 (d), 35.3-38.3 (e)	42.03-43.26*, 39.0-43.0 (a), 34.5-42.5 (b), 33.0-43.0 (c), 42.0 (e), 40.8-42.7 (f)	43.79-46.09*, 47.0 (2), 45.0-48.0 (a), 39.0-48.0 (c), 41.1-48.9 (d), 45.5-46.9 (e)	38.89-40.66*, 39.8-42.1 (3), 38.0-42.0 (a), 39.0-42.0 (c), 38.0-42.0 (d), 38.7-41.3 (e)
E	9.57-11.31*, 17.0-19.0 (1), 18.5-21.0 (a), 14.0-17.0 (d), 16.0-18.0 (e)	9.71-11.59*, 15.0-17.5 (a), 16.5 (e),15.2-15.5 (f)	11.54-15.86*, 17.5-20.0 (a), 15.5-19.5 (b), 19.0-22.0 (e)	13.05-14.84*, 15.0-18.0 (a), 15.5-19.5 (e)
TB	16.83-17.83*, 15.0-17.0 (d), 14.3-18.0 (e)	16.58-19.66*, 16.8 (e), 16.4-16.8 (f)	19.85-20.67*, 17.7-22.6 (d), 20.0-22.0 (e)	15.31-16.40*, 15.5-17.0 (e)
HF	5.71-6.36*, 8.0 (1)	5.25-7.16*	5.82-7.89*	5.38-6.24*
TVL	21.35-24.45*, 24.0-29.0 (1), 24.0-30.0 (a), 24.0-28.0 (d), 21.0-32.0 (e)	20.68-24.67*, 22.0-26.0 (a), 27.0 (e), 21.9-22.3 (f)	28.23-33.67*, 27.0-31.0 (a), 29.0-35.0 (e)	14.97-21.06*, 19.0-24.0 (a), 19.0-25.0 (e)
W	4.0-5.0*	5.0-7.5 (a)	7.0-8.5 (a)	5.0-7.5 (a)
GSL	14.75-15.08*, 16.1-16.3 (1), 15.42-15.79 (e)	17.84-18.70*, 17.82 (e), 17.80-18.41 (f)	18.66-18.90*, 19.14-19.72 (e)	15.97-16.61*, 16.19-16.49 (e)
CBL	11.91-12.40*	14.11-15.81*	15.05-16.17*	13.08-14.11*
IOW	2.15-2.26*, 2.4-2.7 (1)	2.70-3.05*	2.80-3.05*, 3.00 (2)	2.15-2.70*, 2.40 (3)
MT	3.49-3.59*, 5.00-5.20 (1), 5.10 (3), 4.8-5.5 (b), 5.12-5.38 (e)	4.50-5.16*, 6.10 (e), 5.04-6.12 (f)	4.92-5.36*, 6.50 (2), 6.44-6.70 (e)	4.14-5.09*, 5.30-5.40 (3), 5.31-5.42 (e)
C-C	3.14-3.16*, 2.82-3.34 (e)	3.16-3.94*, 3.45 (e), 3.10-3.18 (f)	3.19-3.70*, 3.64-3.85 (e)	3.34-3.64*, 3.80-3.90 (3), 3.39-3.48 (e)
M-M	5.00-5.15*, 5.29-5.43 (e)	5.20-5.97*, 6.11 (e), 5.72-5.82 (f)	5.31-5.69*, 6.06-6.13 (e)	5.60-5.87*, 6.00-6.10 (3), 5.98-6.28 (e)

1 = Philips (1967), 2 = Tate (1941), 3 = Hill and Zubaid (1989), a = Payne *et al.* (1985), b = Corbet and Hill (1992), c = Koopman (1994), d = Kingston *et al.* (2006), e = Suyanto and Struebig (2007), f = Abdullah *et al.* (2003). FA = forearm length, E = ear length, TB = tibia length, HF = hind foot length, TVL = tail length, W = weight, GSL = greatest length of skull, CBL = condylobasal length, IOW = interorbital width, MT = maxillary toothrow length, C-C = canine width, M-M = molar width.

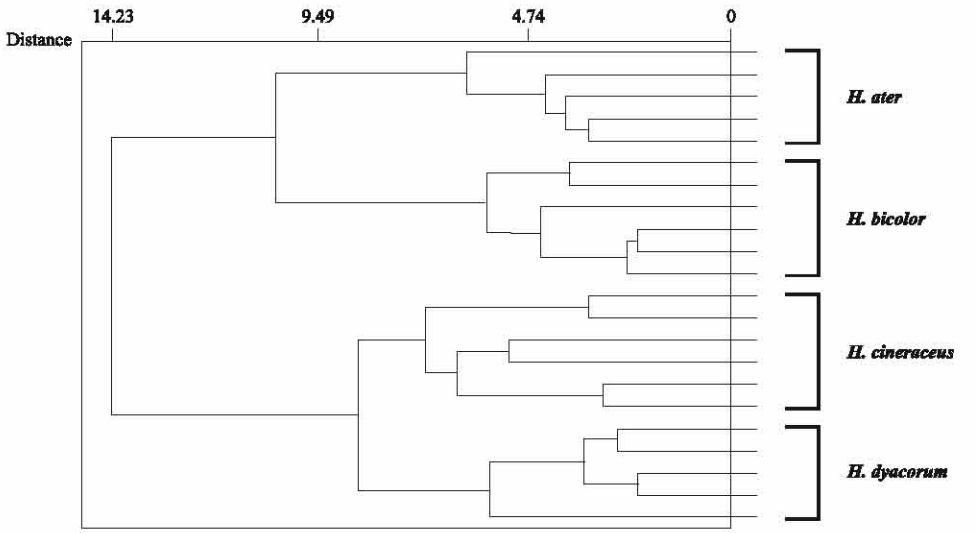

Figure 2. Morphometric hierarchical Cluster Analysis of four *Hipposideros* species (adapted from Sazali, 2009).

recognised as *H. cineraceus* from Peninsular Malaysia (Sazali, 2009; Sazali *et al.*, 2011). Genetic divergences of 10.8% between *H. cineraceus* and *H. ater*, 11.0% between *H. cineraceus* and *H. bicolor* and 8.8% between *H. cineraceus* and *H. dyacorum* (Sazali *et al.*, 2011) provided a good species level discrimination between studied *bicolor* species group individuals. Specimens from Mount Murud showed more than 5% divergence (above normal mammalian species level divergences) (Bradley & Baker, 2001), with other *H. ater* from a different locality. These samples also clustered within other *H. cineraceus* from Borneo with less than 2% divergence, supporting the recognition of *H. cineraceus*.

Thus, we recognised the specimens from Mount Murud as *H. cineraceus* (Ashy roundleaf bat) (Figure 3). Similarities of morphological features, such as the absence of lateral leaflet and simple noseleaves between *H. cineraceus* and *H. ater* (Payne *et al.*, 1985), were further discussed by Hill (1963) and later reviewed by Hill & Francis (1984). *Hipposideros cineraceus* are distributed in Pakistan, northern India, Burma, Thailand, Vietnam, Peninsular Malaysia, Borneo, Sumatra, Krakatau Island, Riau Island, Kangean Island and Luzon Island (Corbet & Hill, 1992). Andersen (1918) includes the Borneon distribution for *H. cineraceus* without a precise locality. The first published record of the Borneon record was described by Phillips (1967) from tidal caves of Marudu Bay (Northwestern Sabah) and Tanjung Berungus (Sabah). In Sarawak, this species was recorded from Bako National Park (unpublished record) and Jambusan Cave (Jub *et al.*, 2003). Both of these studies have identified their specimen based on morphology and none of them were compared morphologically or genetically with other *bicolor* species group individuals. Recent surveys from Sangkulirang peninsula (East Kalimantan) also have reported the occurrence of this bat species in the forest understorey at the karsts formation and near cave entrances (Suyanto & Struebig, 2007). Similarly, their study also experienced difficulties in

discriminating *H. ater* from *H. cineraceus* using external morphology measurements alone.

Figure 3. *Hipposideros cineraceus* Blyth, 1853 - Ashy Roundleaf bat (Photograph by Horn Professor Robert J. Baker, Texas Tech University, USA).

Genetic divergences of 4.3% to 4.8% between specimens from Mount Murud to those from Peninsular Malaysia and Sabah, suggest that the specimens referred to as *H. cineraceus* in this study may have an independent evolutionary lineage from others and probably have separated for enough time to speciate (Bradley & Baker, 2001). Cyt *b* phylogenetic analyses showed a reciprocal monophyly for this taxonomic unit, indicating historically isolated and independently evolving sets of populations. This suggests the recognition of the Mount Murud population as an Evolutionary Significant Unit (Moritz, 1994). Further analyses including nuclear gene with broader sampling could provide the basis for prioritising conservation effort for this taxonomic groups.

In Sarawak, *H. cineraceus* is listed as a protected animal under the First Schedule (Section 2[1]) Part II of the Sarawak Wild Life Protection Ordinance 1998. As this new distribution record was counted at a collecting site that has been granted for a large scale logging project, we urge the government and relevant authorities to reevaluate the numbers of approved permits, and suggest appropriate logging sites at Mount Murud to avoid further destruction of this population's habitat. We propose future biodiversity assessments and surveys should include more data (e.g. cranial measurements, molecular data) to increase the accuracy of bats species identification. Such an approach is important to properly recognise species and document the rich diversity found in Malaysia.

ACKNOWLEDGEMENTS

We would like to acknowledge the IRPA grant 09-02-09-1022-AE001 awarded to MTA, YE & AAS and Faculty of Resource Science and Technology, UNIMAS for financial and administrative support. We thank Mr. Mohd. Jalani Mortada and Ms. Audrey Mengan Jackson Bundan for their field assistance and companionship during this survey. We also thank Horn Professor Dr. Robert J. Baker of Texas Tech University for sharing the photograph presented here. Senior author SNS was supported by the Ministry of Science, Technology and Environment, Malaysia and FAAK's study at Texas Tech University was supported by the Ministry of Higher Education, Malaysia and UNIMAS.

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